Effects of the NMDA Receptor Antagonists on Deltamethrin-Induced Striatal Dopamine Release in Conscious Unrestrained Rats

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ABSTRACT. To better understand the neurotoxicity caused by the pyrethroid pesticide, we examined the effects of the N-methyl-D-aspartate (NMDA) receptor antagonists MK-801, a non-competitive cation channel blocker, and 2-amino-5-phosphonovaleric acid (APV), a competitive Na+ channel blocker, on extracellular dopamine levels in male Sprague-Dawley rats receiving the type II pyrethroid deltamethrin using an in vivo microdialysis system. Deltamethrin (60 mg/kg, i.p.) evidently increased striatal dopamine levels with a peak time of 120 min, and the local infusion (i.c.) of either MK-801 (650 μM) or APV (300 μM) completely blocked these actions. The fluctuation in the dopamine metabolite 3-MT also resembled that in dopamine. Our results suggest that dopamine-releasing neurons would be modulated via the NMDA receptor by the excitatory glutamatergic neurons after deltamethrin treatment.

KEY WORDS: deltamethrin, microdialysis, rat, striatal dopamine.

NOTE. Toxicology

Pyrethroids have been chemical-structurally classified into two groups; type I pyrethroids (e.g. allethrin and pyrethrin) lack a cyano moiety, and type II pyrethroids (e.g. deltamethrin and cyhalothrin) possess a cyano group in the α-position [6]. However, these classifications have several shortcomings, because common toxic syndromes in mammals are different for the respective types [4, 6, 11]. Since type II synthetic pyrethroid esters have exhibited a highly selective toxicity without leaving a residue, they have been widely used as a pesticide [6]. As the pharmacological action of the pyrethroids including deltamethrin, it has been shown that they modify the gating kinetics of axonal Na+ channels involved in the inward flow of Na+ ions, producing the action potential in cells that are normally closed at the resting potential [6, 8–10]. Most recently, it has been reported that deltamethrin stimulates the glutamate receptor, or blocks the γ-aminobutyric acid (GABA_A) receptor [5], presumably leading to chronic seizures [6]. In the present work, to better understand the neurotoxicity induced by the pyrethroid, we used an in vivo microdialysis system to examine the effects of the N-methyl-D-aspartate (NMDA) receptor antagonists MK-801, a non-competitive cation channel blocker, and 2-amino-5-phosphonovaleric acid (APV), a competitive Na+ channel blocker, on extracellular dopamine levels in conscious unrestrained male rats receiving the type II pyrethroid pesticide deltamethrin. Although pyrethroids were found to show differential effects on the nervous system [6] as mentioned above, a high dose of deltamethrin has been thought to increase extracellular dopamine levels during the late response phase [4]. The NMDA receptor, which is an ionotropic receptor that is modulated by voltage-dependent Mg2+ blockade and possesses a Ca2+ channel, is one of the excitatory glutamate receptors [7]. Additionally, the NMDA receptor is likely related to the long-term potentiating action of dopamine [4, 7].

Male Sprague-Dawley (SD) rats weighing 220–290 g (7–10 weeks of age) obtained from Japan SLC Laboratory (Hamamatsu, Japan) were used in this investigation. The animals were housed in plastic cases in a ventilated room with a controlled temperature (23 ± 2°C), relative of humidity (55 ± 22%) and 12-hr light/dark cycle of the artificial light. They were provided a standard commercial laboratory chow (MEQ, Oriental Yeast, Co., Tokyo, Japan) and tap water ad libitum throughout the study. Animal handling and procedures were approved by the Animal Experimental Ethics Committee of Iwate University. Deltamethrin hydrochloride, MK-801 and APV were purchased from Wako Pure Chemical Industries (Osaka, Japan). Other reagents utilized were of the best analytical grade.

The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and placed in a stereotaxic apparatus (DKI-900, Kopf Instruments, Tokyo). Then, a guide cannula (AG-8, Eicom, Kyoto, Japan) was carefully implanted into the striatum. The appropriate placement of the cannula was confirmed in advance by histochemical staining of the dopaminergic nerves. After surgery, the animals were returned to their home cages and allowed to recover for at least 3 days before the experiment. The extracellular levels of dopamine and its metabolites (DOPAC, HVA and 3-MT) were determined by the in vivo microanalysis method [4] using a high-performance liquid chromatography (HPLC) system equipped with an electrochemical detector (HPLC-ECD, Eicom). Each sample was measured in duplicate, and its average was calculated. The retention times of dopamine, DOPAC, HVA and 3-MT were 8.8, and 6.1, 13.7 and 19.5 min, respectively.

Deltamethrin (30 mg) was dissolved in dimethyl sulfoxide (DMSO, 50 μl) and Tween 20 (15 μl), and then made up...
to 60 mg/ml with distilled water. The deltamethrin solution was administered intraperitoneally at a dose of 60 mg/kg to groups of 4 animals each. Control animals (n=4) were given the vehicle alone (DMSO, Tween 20 and distilled water) in the same way as deltamethrin-treated animals. MK-801 and APV were dissolved in Ringer’s solution at final concentrations of 650 μM and 500 μM, respectively, and they were infused intracisternally at an injection speed of 2 μl/min via a microdialysis tube immediately after deltamethrin or vehicle treatment. The dosage level of the respective test compounds utilized was selected based on the results of the previous reports [2, 4, 5]. When the basal efflux of striatal dopamine, DOPAC, HVA and 3-MT was examined at 20-min intervals for 120 min before deltamethrin or vehicle treatment, the basal efflux values of 0.27 ± 0.5, 93.9 ± 10.5, 63.7 ± 9.0 and 0.20 ± 0.02 pg/μl (mean ± SEM, n=4), respectively, were recorded. The results were represented as a percentage of the mean basal efflux of dopamine and its metabolites. The area under the curves from 0 to 180 min (AUC0–180 min) was calculated with a linear trapezoidal rule.

Data are expressed as the means ± standard errors (SEM). Statistically significant differences were detected using one-way analysis of variance (ANOVA), followed by Student’s t-test for two groups, and by Tukey-Kramer test for multiple comparisons. The threshold for statistical significance was more than p<0.05.

In rats given deltamethrin (60 mg/kg, i.p.) alone, their toxic signs included CS syndrome (choreoathetosis and salivation), tremor, chronic seizure and/or staggering gait. These signs appeared from about 5 min after injection, and continued until 180 min. Striatal dopamine release increased gradually from 60 min after injection, peaked at 120 min, and remained at high levels until 180 min (Fig. 1). These results were almost consistent with those of the previous study [4], although no significant decrease in striatal dopamine release was noted at 40 min. In either early or late response phase, therefore, no relationship was seen between appearance of the toxic signs and dopamine release. Neither effect on clinical signs nor dopamine releases was observed after treatment with vehicle, MK-801 alone or APV alone. In local infusion of either MK-801 or APV to rats receiving deltamethrin, the toxic signs were not ameliorated. Meanwhile, striatal dopamine release in the late phase from 90 to 120 min was completely blocked (Fig. 1), and its AUC0–180 min values were significantly reduced (Fig. 2). These findings indicate that the NMDA receptor is involved in striatal dopamine releases evoked by deltamethrin, and that Na+ channels and cation channels (most likely Ca2+ channels) play a crucial role in this event. Previously, Hossain et al. [4] have postulated that the type II pyrethroids may be acting on striatal dopamine release via multiple mechanisms including dopaminergic circuitry. Our results were considered to support at least in part their hypothesis. Among the striatal dopamine metabolites, deltamethrin increased only in 3-MT levels, but not in DOPAC or HVA levels. The AUC0–180 min values for 3-MT resembled those for dopamine, and elevated 3-MT levels were completely blocked by the local infusion of MK-801 or APV (Fig. 2). Since 3-MT was produced from dopamine via catechol-O-methyltransferase (COMT) on the postsynaptic membrane, increased 3-MT levels may also reflect an elevation in dopamine releases. However, this result was somewhat different from that of Hossain et al. [4], who suggested that deltamethrin did not modulate the release of dopamine metabolites in the striatum. This discrepancy may be explained by different compositions (DMSO containing ethanol in their study versus DMSO in our study) of the vehicle. Ethanol has been believed to exert various effects on the function of the NMDA receptors [1]. Alternatively, further studies are required to resolve this matter. Recently, since dopamine

**Fig. 1.** Effects of MK-801 (left) or APV (right) on striatal dopamine levels in conscious unrestrained male SD rats receiving a single intraperitoneal injection of deltamethrin (Delta) at 60 mg/kg. MK-801 (650 μM) or APV (500 μM) was infused intracisternally at an injection speed of 2 μl/min via a microdialysis tube immediately after deltamethrin or vehicle treatment. The values show the mean ± SEM of 3–4 animals. †† p<0.01 vs. vehicle control. ** p<0.01 vs. Delta alone. Among four groups in each study (left or right), significantly increased dopamine level was seen in the Delta alone group (††). Between Delta alone and Delta plus MK-801 groups or Delta alone and Delta plus APV groups, significantly decreased dopamine level was noted in the Delta plus MK-801 or Delta plus APV group (**).
released has been reported to affect AMPA receptors as well as NMDA receptors existing in the dopaminergic nerve terminals [12, 13], additional investigations are also needed in this point.

Under the conditions of this study, the fact that striatal dopamine release was completely blocked by either treatment with 650 μM of MK-801 or 500 μM of APV demonstrates that both antagonists own much the identical affinity to the NMDA receptors, suggesting an equal potential in the control of dopamine release.

Taken together with present and previous data, it was strongly suggested that deltamethrin-induced striatal dopamine release in the late phase largely involved the NMDA receptors in addition to the Na⁺ channels and voltage-dependent Ca²⁺ channels. As their proposed mechanisms, it was implied that deltamethrin acted on both dopaminergic and glutamatergic neurons, consequently eliciting depolarizing potentials by opening Na⁺ channels in the dopaminergic neurons, and alleviating the voltage-dependent Mg²⁺ blockage of NMDA receptors, followed by increased Ca²⁺ influx. The Ca²⁺ influx would further facilitate dopamine releases due to Ca²⁺ from the voltage-dependent Ca²⁺ channels.

In conclusion, treatment of rats with deltamethrin (60 mg/kg, i.p.) evidently increased striatal dopamine and metabolite 3-MT levels during the late response phase, and the local infusion (i.c.) of either MK-801 (650 μM) or APV (500 μM) completely blocked these actions. Our results suggest that dopamine-releasing neurons would be modulated via the NMDA receptors by the excitatory glutamatergic neurons after deltamethrin treatment.

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REFERENCES


